

TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203CE. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot was not noted. The test sample is FC-203CE. Current information indicates it is a mixture of 1.84% PFOS, 30% diethylene glycol butyl ether, 60.23% water, 2.56% Sultone foamer, 3.1% sodium octyl sulfate, 2.1% polyoxyethylene monooctylphenyl ether, 0.12% sodium lauryl sulfate, and 0.05% tolyltriazole.

The following summary applies to a mixture with incompletely characterized concentrations of impurities. Data may not accurately reflect the toxicity of the fluorochemical component of the test sample.

METHOD:

Method: OECD 201 and EPA-600/9-78-018

Type: Acute Static

GLP: No

Year study performed: 1991

Species: *Selenastrum capricornutum*

Source: In-house culture. Originally from the Culture Collection of Algae at the University of Texas at Austin.

Element basis: Algal cell counts (cells/ml)

Exposure period: 96 hours

Statistical Methods: Performed using standard statistical techniques.

Stephan, 1983. NOEC was calculated using Dunnett's test.

Analytical monitoring: pH and temperature

Test Conditions:

Algal Nutrient Medium: Nutrient medium per U.S. EPA, 1978 prepared in deionized water. This nutrient medium provided all mineral nutrients essential for algal growth and also served as the diluent for all algal operations. The pH of this synthetic algal medium was ~8.0.

Stock and test solution preparation: A 1000 mg/L primary stock solution was prepared in algal medium. Aliquots were added directly to algal medium in test vessels to create test solutions.

Exposure vessels: 250 mL Erlenmeyer flasks containing 100 mL test solution and capped with inverted glass beakers.

Agitation: Continuous rotary shaker, rate of rotation not given.

Number of replicates: 3

Initial cell loading: 1.0×10^4 cells/mL.

Number of concentrations: five plus a blank control

Lighting: Continuous lighting at 50 $\mu\text{Ein}/\text{sec}/\text{m}^2$ using cool-white fluorescent lamps.

Water Chemistry:

pH range (0 – 96 hours):

8.2 – 9.9 (control exposure)

8.3 – 8.6 (1,000 mg/L exposure)

Test temperature range (0 – 96 hours):

23.0 – 23.4°C (incubator)

RESULTS

Nominal concentrations: Blank control, 150, 250, 400, 600, and 1000 mg/L

Algal Growth Response at 96 Hours

Nominal Exposure Conc. (mg/L)	Avg. Cell-Count (cells/mL)	Avg. Specific Growth rate (μ)	% Change ⁽¹⁾
Control	479,000	0.040	—
150	188,000	0.031	22
250	58,000	0.018	55
400	20,000	0.007	82
600	3,000	-0.012	100
1000	0	0.000	100

(1) Average growth rate in control compared to test concentration growth rate.

Element values: 96-hour E_rC_{50} = 230 (212 – 248) mg/L

96-hour NOEC = <150 mg/L

Element values are based on nominal concentrations.

Reversibility of Growth Inhibition: Not determined.

Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical proportion alone.

CONCLUSIONS

The test substance exhibits a 96-hour EC_{50} of 230 mg/L with a 95% confidence interval of 212 to 248 mg/L. The 96-hour No Observed Effect Concentration (NOEC) was <150 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

This test was conducted by EnviroSystems Division of Resource Analysts, Incorporated, of Hampton, NH at the request of the 3M Company, Lab Request number J1884-1, 1991.

OTHER

Last changed: 6/26/00

Study Title

Static Acute Toxicity of FC-203CE
to the Alga, *Selenastrum capricornutum*

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Study Completed

July, 1991

Performing Laboratory

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I. SUMMARY

The acute toxicity of FC-203CE to the alga, *Selenastrum capricornutum*, is described in this final report. The test was conducted for 3M Company for 96 hours during July 8 to July 12, 1991, at the EnviroSystems Division of Resource Analysts, Inc. in Hampton, New Hampshire. It was conducted by Jeanne Hagazu, Peter Kowalski, Ellen Stanford, Robert Boeri, and Timothy Ward.

The test was performed under static conditions with five concentrations of test substance and a dilution water control at a mean temperature of 23.2°C. The dilution water was synthetic media prepared with sterile deionized water. Flasks were incubated on a rotary shaker. Light was adjusted to 50 $\mu\text{Ein}/\text{sec}/\text{m}^2$ with a photoperiod of 24 hours light and 0 hours dark. Nominal concentrations of FC-203CE were: 0 mg/L (control), 150 mg/L, 250 mg/L, 400 mg/L, 600 mg/L, and 1,000 mg/L. Nominal concentrations were used for all calculations.

Algae used in the test was from an in-house culture that was maintained under test conditions. Exposure of algae to the test substance resulted in a 72 hour EC50 of 340 mg/L FC-203CE and a no observed effect concentration (NOEC) of 150 mg/L. The 96 hour EC50 and NOEC are 230 mg/L and less than 150 mg/L, respectively.

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IV. INTRODUCTION

This study was sponsored by 3M Company, St. Paul, Minnesota. The objective of the study was to determine the acute toxicity of FC-203CE to a freshwater alga. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation. The report also contains an appendix that presents the water quality data collected during the test.

V. METHODS AND MATERIALS

TEST SUBSTANCE:

FC-203CE (EnviroSystems Sample Number 2935A) was delivered to EnviroSystems on May 23, 1991. It was contained in a 40 oz. plastic bottle that was labelled with the following information: "J1884-1, FC-203CE". FC-203CE (an orange-brown liquid) was supplied by 3M Company, 935 Bush Avenue, St. Paul, Minnesota. Prior to use the test material was stored in the dark at room temperature. A reserve sample (approximately 1.1 grams) will be retained at EnviroSystems for a minimum of 10 years.

DILUTION WATER:

Water used for acclimation of test organisms and for all toxicity testing was sterile enriched media (U.S. EPA, 1978) with a pH of approximately 8.0. Results of chemical analysis of a representative sample of deionized water used to prepare the media are presented in Table 1.

TEST ORGANISM:

Algae used for the test was from a culture originally procured from the Culture Collection of Algae at the University of Texas at Austin and delivered to EnviroSystems on May 14, 1991. The identity of the culture was verified using an appropriate taxonomic key. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions.

TOXICITY TESTING:

The definitive toxicity test was performed during July 8 to 12, 1991. It is based on procedures of the OECD (1984). The test was conducted at a target temperature of $22 \pm 2^\circ\text{C}$ with five concentrations of test substance and a dilution water control. A 1,000 mg/L stock solution was prepared in dilution water. The stock solution was added directly to dilution water contained in the test vessels without the use of a solvent. Nominal concentrations of the test material were: 0 mg/L (control), 150 mg/L, 250 mg/L, 400 mg/L, 600 mg/L, and 1,000 mg/L.

Table 1. Chemical characterization of a representative sample of deionized water used to prepare test media for toxicity test

Parameter	Unit of Measurement	Reporting Limit	Measured Value
Organochlorine pesticides	ug/L	2	ND
Organophosphorus pesticides	ug/L	0.5	ND
Polychlorinated biphenyls	ug/L	0.5	ND

Note: ND = not detected at or above the reporting limit.

Algae was randomly and equally distributed among three replicates of each treatment at the rate of 10,000 cells/ml. The test was performed in 250 ml glass Erlenmeyer flasks that contained 100 ml of test solution. The flasks were capped with inverted glass beakers. Test vessels were randomly arranged on a rotary shaker in an incubator during the 96 hour test (a random numbers table was used to select the location of each vessel). A 24 hour light and 0 hour dark photoperiod was maintained with cool-white fluorescent lights that provided a light intensity of $50 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

The number of algal cells/ml in each test vessel was determined visually every 24 hours by means of direct microscopic counts with a hemacytometer. The pH (Beckman model PHI 51 meter) was determined in each test flask at the beginning and end of the test, and temperature (ASTM mercury thermometer) was measured and recorded daily in the incubator.

STATISTICAL METHODS:

Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The binomial or moving average method was used by the author to calculate EC50 values using nominal concentrations of test substance. The NOEC was calculated using Dunnett's test, which includes a parametric one-way analysis of variance (ANOVA). The average specific growth rate was calculated as the natural log of the number of cells per ml at 24, 48, 72, or 96 hours minus the natural log of the number of cells per ml at 0 hours divided by the exposure period. The percent change from control is calculated by subtracting the sample average specific growth rate from the control average specific growth rate, dividing that value by the average specific growth rate in the control and multiplying that value by 100. The percent change from control was used to compute EC50 and NOEC values.

VI. RESULTS

Biological and water quality data generated by the acute toxicity test are presented in Table 2 and Appendix A, respectively, and the percent of control and average specific growth rate information is presented in Table 3.

The 24, 48, 72, and 96 hour EC50s for algae exposed to FC-203CE are presented in Table 4. The 72 hour EC50 is 340 mg/L, and the 72 hour NOEC is 150 mg/L FC-203CE. The 96 hour EC50 is 230 mg/L and the 96 hour NOEC is less than 150 mg/L.

Table 2. Growth data from toxicity test

Nominal Concentration (mg/L)	rep.	Number of Cells per Milliliter x 10 ³ (hour)				
		0	24	48	72	96
0.0 (control)	1	10	12	120	144	372
	2	10	18	106	218	450
	3	10	18	136	196	615
	mean	10	16	121	186	479
150	1	10	14	64	88	215
	2	10	14	64	110	195
	3	10	10	70	70	155
	mean	10	13	66	89	188
250	1	10	20	46	50	50
	2	10	20	46	85	80
	3	10	20	48	75	45
	mean	10	20	47	70	58
400	1	10	14	54	25	25
	2	10	10	40	40	10
	3	10	16	46	25	25
	mean	10	13	47	30	20
600	1	10	30	64	40	10
	2	10	22	38	20	0
	3	10	20	48	20	0
	mean	10	24	50	27	3
1,000	1	10	24	38	25	0
	2	10	22	30	35	0
	3	10	20	36	20	0
	mean	10	22	35	27	0

Table 3. Percent change from control and average specific growth rate from toxicity test

Nominal Concentration of Test Substance (mg/L)	Average specific growth rate				Percent change from control			
	24hr	48hr	72hr	96hr	24hr	48hr	72hr	96hr
0.0 (control)	0.020	0.052	0.041	0.040	0	0	0	0
150	0.011	0.040	0.030	0.031	45	23	27	22
250	0.029	0.032	0.027	0.018	-45	38	34	55
400	0.011	0.032	0.015	0.007	45	38	63	82
600	0.037	0.034	0.014	-0.012	-85	35	66	100
1,000	0.033	0.026	0.014	0.000	-85	50	66	100

Table 4. Median effective concentrations (EC50s) from toxicity test

Exposure period	EC50	95 percent confidence limits	Calculation method
24 hours	> 1,000 mg/L	--	--
48 hours	< 150 mg/L	--	--
72 hours	340 mg/L	247 - 438 mg/L	Moving Average
96 hours	230 mg/L	212 - 248 mg/L	Probit

VII. REFERENCES

OECD. 1984. Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 201, Alga, Growth Inhibition Test. Adopted June 4, 1984.

Stephan, C.E. 1983. Computer program for calculation of LC50 values. Personal communication.

U.S. EPA. 1978. The *Selenastrum capricornutum* Printz Algal Assay Bottle Test. EPA-600/9-78-018. July, 1978.

Appendix A. WATER QUALITY DATA FROM TOXICITY TEST

Table A.1. Temperature measured during toxicity test

Day of Exposure	Temperature of Incubator, °C
0	23.2
1	23.0
2	23.0
3	23.4
4	23.2

Table A.2. pH measured during toxicity test

Nominal concentration (mg/L)	Replicate	pH	
		initial	final
0 (control)	1	8.2	9.8
	2	8.2	9.8
	3	8.2	9.9
150	1	8.4	8.7
	2	8.4	8.7
	3	8.4	8.7
250	1	8.4	8.7
	2	8.4	8.7
	3	8.4	8.7
400	1	8.4	8.6
	2	8.4	8.6
	3	8.4	8.6
600	1	8.4	8.6
	2	8.4	8.6
	3	8.4	8.6
1,000	1	8.3	8.6
	2	8.3	8.6
	3	8.3	8.6